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Effect of Bovine Manure on Fecal Coliform Attachment to Soil and Soil Particles of Different Sizes[▽]

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Manure-borne bacteria can be transported in runoff as free cells, cells attached to soil particles, and cells attached to manure particles. The objectives of this work were to compare the attachment of fecal coliforms (FC) to different soils and soil fractions and to assess the effect of bovine manure on FC attachment to soil and soil fractions. Three sand fractions of different sizes, the silt fraction, and the clay fraction of loam and sandy clay loam soils were separated and used along with soil samples in batch attachment experiments with water-FC suspensions and water-manure-FC suspensions. In the absence of manure colloids, bacterial attachment to soil, silt, and clay particles was much higher than the attachment to sand particles having no organic coating. The attachment to the coated sand particles was similar to the attachment to silt and clay. Manure colloids in suspensions decreased bacterial attachment to soils, clay and silt fractions, and coated sand fractions, but did not decrease the attachment to sand fractions without the coating. The low attachment of bacteria to silt and clay particles in the presence of manure colloids may cause predominantly free-cell transport of manure-borne FC in runoff.

Manure is the primary source of pollution from animal feeding operations. There are an estimated 376,000 livestock operations in the United States, which generate 58.1 million tons of manure each year. According to the 1998 National Water Quality Inventory, approximately 60% of the pollution in rivers and 45% in lakes come from agricultural sources (12). Animal feces are deposited on land by grazing animals, and livestock manure and wastes from processing facilities are increasingly applied on agricultural soils in the form of solid or liquid slurries as fertilizers for silage, grazing, or crop production. Livestock manures/wastes may contain pathogenic bacteria, such as *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella* spp., and certain strains of *Escherichia coli*, which may be released into the environment in large numbers (20, 27, 35, 43, 45). Sixty-five outbreaks of human infections linked to water have been reported in the United Kingdom in 1991 to 2000 (22), while 230 outbreaks have been reported in the United States in 1991 to 1998 (7).

The transport of manure-borne pathogenic organisms in overland flow may be a significant cause of surface water contamination (6, 11, 25, 40). Numerous experimental studies indicate that overland flow can transport substantial amounts of fecal bacteria on steep pastoral land (4, 11, 41), grazed pastures (11, 40, 43), or grassland and crops receiving cattle slurry (21, 34). Curriero et al. (8) have reported statistically significant correlations between the increase in heavy rainfall events and waterborne disease outbreaks in the United States for the period 1948 to 1994.

It is generally recognized that bacteria in runoff can be transported as free cells or cells attached to soil particles, to fragments of vegetation and residue, and to manure particles (14, 23, 33, 44). Relatively little is known about the transport rates of free cells versus those of attached cells, which are likely to differ substantially due to differences in particle sizes and densities. Coyne et al. (5) studied the effect of sediment settling time on fecal coliform (FC) concentrations in runoff water from grass filter strips. They observed a 10-fold decrease of FC concentrations in runoff water after the silt fraction had settled as compared to the settling sand fraction, which suggested much greater attachment to the silt fraction. Jeng et al. (24) studied *E. coli* attachment to five sediment fractions in fresh storm water. They found that approximately 80%, 18%, and 2% of attached *E. coli* cells were associated with the silt fraction, the clay fraction, and the sand fraction, respectively. Bengtsson and Ekere (1) have studied attachment of indigenous groundwater bacteria to five fractions and two mixtures of a sandy soil taken at an infiltration field. They did not find correlations between the partitioning coefficient and particle size or specific surface area. Karpinskaya (quoted in reference 27) showed that the percentage of *Serratia marcescens* cells attached to quartz particles increased as the particle size decreased from 1,000 μm to <1.5 μm. The percentage of *S. marcescens* cells attached to the sand fraction was 13 to 29 times less than the percentage attached to the clay-sized fraction and 5 to 25 times less than the percentage attached to the silt-sized fraction.

We are not aware of any previous studies of bacterial attachment to soil particles of different sizes as affected by the presence of manure particles. The objectives of the present work were to compare levels of FC attachment to soil particles of different sizes and to assess the effect of bovine manure on FC attachment to soil and soil particles.

MATERIALS AND METHODS

The attachment of FC to the soils and soil fractions consisting of particles of different sizes was studied in batch experiments. Loam soil samples were taken

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TABLE 1. Selected soil properties

Soil type	Particle density ($Mg\ m^{-3}$)	Soil fraction or OC content (%)				pH
		Clay	Silt	Sand	OC	
Tyler loam	2.560	26.4	45.8	27.8	3.3	5.61
BARC SCL	2.611	20.3	26.7	53	1.7	5.57
BARC loam	2.552	27.0	36.7	36.3	2.5	4.65

TABLE 2. OC content in soil fractions

Soil	OC content (%) in:				
	Clay	Silt	Sand (particle size [mm])		
			0.0625–0.125	0.125–0.25	0.25–0.5
Tyler loam	3.27	2.82	4.67	4.60	4.68
BARC SCL	3.88	2.63	0.01	0.02	0.0
BARC loam	3.27	2.56	0.14	0.07	0.02

from the A horizon of Tyler soil (fine-silty, mixed, mesic, Eric Fragiaquults) in Franklin County, PA. The soil was under a complex mixture of grasses and legumes. Sandy clay loam (SCL) and loam soil samples were taken from runoff plots (0- to 20-cm depth) at the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Beltsville Agricultural Research Center (BARC). The runoff plots were constructed in 1998 using commercial topsoil and planted with blue fescue (*Festuca ovina* L. 'Glauca') and white clover (*Trifolium repens* L.). The air-dried soil was separated into five fractions of different particle sizes. Three sand fractions were obtained by wet sieving through 0.125-, 0.25-, and 0.5-mm sieves. The silt fraction, with particle sizes of 0.002 to 0.05 mm, and the clay fraction, with particle sizes <0.002 mm, were separated using repeated sedimentation of soil passed through a 0.0625-mm sieve. The soils and soil fractions were kept air dried prior to the experiments and soil property determinations.

The soil textures were measured with the hydrometer method (17) after dispersion with sodium pyrophosphate, $Na_4P_2O_7$. The particle densities were measured with the pycnometer method (15). The soil pH values were measured at a solid-to-liquid ratio of 1:1 (42). The organic carbon (OC) contents were measured in the soil samples and the soil fraction samples with the dry combustion method (32). Selected properties of the soils are given in Table 1. The surfaces of the sand particles were studied using microscopy. Photomicrographs were taken with the aid of a Zeiss Axiomat bright-field microscope (Carl Zeiss, Inc., Baltimore, MD) with a 10× Plan-Neofluar objective and recorded digitally using a Spot-RT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI). Organic matter removal from the sand fractions of the Tyler soil was performed using peroxide digestion (2).

Bovine manure was collected from the Dairy Research Unit of the USDA-ARS facility in Beltsville. The manure contained 15.8% total solids, 1.210 g $liter^{-1}$of total nitrogen, 0.306 g $liter^{-1}$ of ammonium nitrogen, less than 0.001 g $liter^{-1}$ of nitrate, 0.148 g $liter^{-1}$ of soluble phosphorus, and 0.303 g $liter^{-1}$ of total phosphorus. The bovine manure was added to water to obtain water-manure suspensions of 40 g $liter^{-1}$ and then filtered through cheesecloth to separate the suspension from plant residue and bedding straw. The filtered manure was centrifuged at 100 × *g* for 15 min to separate the coarse fractions from the liquid and colloidal fractions. The particle size distribution was measured in the manure using an LA-920 laser-scattering particle size distribution analyzer (Horiba Instruments Inc., Irvine, CA). The average particle size was 2.48 ± 1.48 μm, and the solid/liquid ratio in the centrifuged manure suspension was 0.114 ± 0.001%. The FC concentrations in the manure were determined with a spread-plate method (3). The bacterium-manure suspension in amounts of 50 μl was plated onto MacConkey agar by using an Autoplate 4000 spiral plater (Spiral Biotech, Bethesda, MD) and incubated for 14 h at 44.5°C. The FC CFU were counted using a Protocol plate reader (Synoptics, Cambridge, United Kingdom). The manure suspension was aged for approximately 3 months at a temperature of 4°C until the FC content in the manure decreased to $<10^3$ CFU ml^{-1}.

The aged bovine manure was cultured in a dilute yeast extract broth (0.01% wt/vol) at 37°C for 24 h with the goal of producing an inoculum for attachment studies. The cultured cells were pelleted by centrifugal sedimentation at 6,000 × *g* for 10 min and the pellet was resuspended in deionized water. Serial dilutions of the suspension were prepared and appropriate amounts added to water-manure suspensions to obtain FC concentrations of 10^2, 10^3, 10^4, and 10^5 CFU ml^{-1}. Bacterium-water or bacterium-manure suspensions were added to 2 g of air-dried soil and soil fractions to give a soil-to-suspension ratio of 1:10 in triplicate for each FC concentration. Bacterial and soil suspensions were stirred for 2 h on a rotary shaker at 8°C to minimize microbial growth. It was determined in an initial experiment that 2 h was sufficient to achieve equilibrium between cells in suspension and attached to soil fractions. The suspensions were centrifuged at 100 × *g* for 15 min in 50-ml centrifuge tubes (PTD PRO; Elkay, Mansfield, MA) at 8°C. Soil-free FC suspensions were centrifuged simultaneously with each soil suspension in triplicate to correct for bacterial attachment to centrifuge tube walls. The electrical conductivity (EC), pH, and FC concentrations were measured in the supernatants, applied water, and water-manure suspensions. The EC in suspensions was measured with an MPM 1000 conductivity meter (Solomat Ltd., Bishops Stortford, United Kingdom).

The amount of attached FC was calculated from the difference between the amount applied and the amount recovered in the supernatant.

The linear isotherm equation $S = K_sC$, where S is the equilibrium concentration of CFU per gram of soil (CFU g^{-1}), C is the equilibrium FC concentration in solution (CFU ml^{-1}), and K_s is the partition coefficient (ml g^{-1}), was fitted to the data. Statistical analyses were done using the SPLUS software (Mathsoft, Cambridge, MA).

RESULTS AND DISCUSSION

The properties of the soils and soil fractions are shown in Table 1. The clay content did not differ substantially among the soils, whereas the sand content was 1.5 times less in the BARC loam and 2 times less in the Tyler loam than in the BARC SCL soil (Table 1). The silt and OC contents were higher in the Tyler soil than in the BARC soils. The lowest values of silt and OC content were found in the BARC SCL soil. The OC content in the sand fractions was negligible compared to the content in the silt and clay fractions of the BARC soils and reached the highest value in the clay fractions (Table 2). In the Tyler soil, the OC content in the clay fraction was higher than in the silt fraction but was less than in any of the sand fractions. The pH values were less than 6 in all soils and were higher in the Tyler soil and the BARC SCL than in the BARC loam soil (Table 1). The microscopic study revealed the presence of a coating on the surface of the Tyler sand, while this coating was absent from the BARC sand fractions. The surfaces of the sand fractions in the Tyler soil had a black-matted, rough appearance (Fig. 1a), while the sand fractions of the BARC soils were glossy and all sand particles were translucent (Fig. 1b and c). After applying the organic matter removal treatment to the Tyler sand, the coating partially disappeared. The organic matter content in the Tyler sand decreased approximately twofold after the organic matter removal treatment.

All soil-bacterium suspensions had pH values of less than 7 and EC values of less than 0.2 $S\ m^{-1}$ (Fig. 2a and 3a). Analysis of variance showed that the fraction size was the significant factor ($P < 0.05$) of the values of both pH and EC in all suspensions except for the EC values in the Tyler soil. Overall, the pH and EC values were higher in the clay and silt fractions than in the sand fractions of the BARC soils (Fig. 2a and 3a), and the pH values were higher in the BARC SCL than in the BARC loam soil.

Addition of bovine manure increased the pH and EC values in the suspensions. The pH values were found to be in the range from 7 to 8 (Fig. 2b) and the EC values were in the range from 1.1 to 1.4 $S\ m^{-1}$ in the manure-soil-bacterium suspensions (Fig. 3b). The increases in pH from the values in manure-

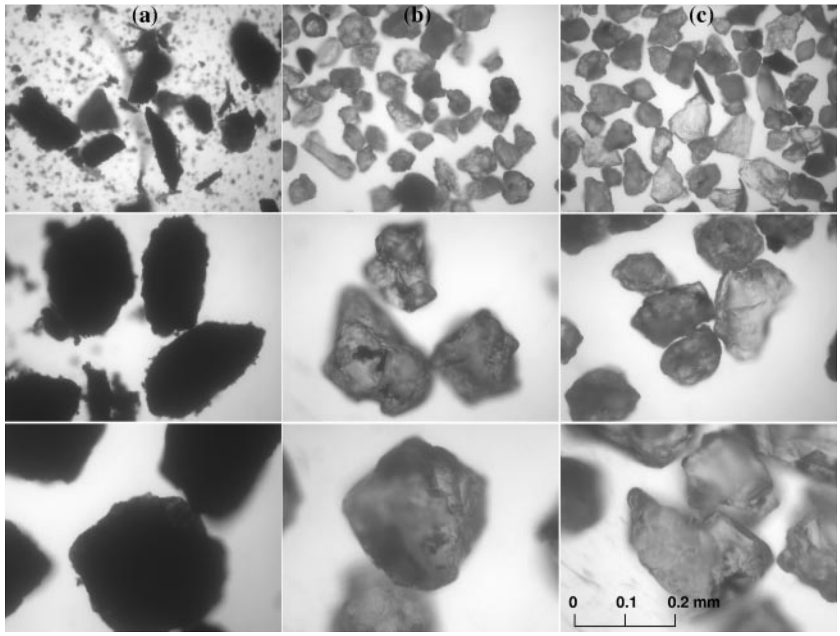

FIG. 1. Coated (Tyler soil) (a) and uncoated (BARC soils) (b, c) sand fraction surfaces.

free suspensions were more pronounced for the BARC loam soil and soil fractions and less pronounced for the Tyler soil fractions. The increase in EC was greater in the fractions of the BARC soils than in the fractions of the Tyler soil. Analysis of variance showed that the fraction size was the significant factor ($P < 0.05$) of the values of both pH and EC in all suspensions with manure except for the EC value in the Tyler soil and the pH value in the BARC loam soil.

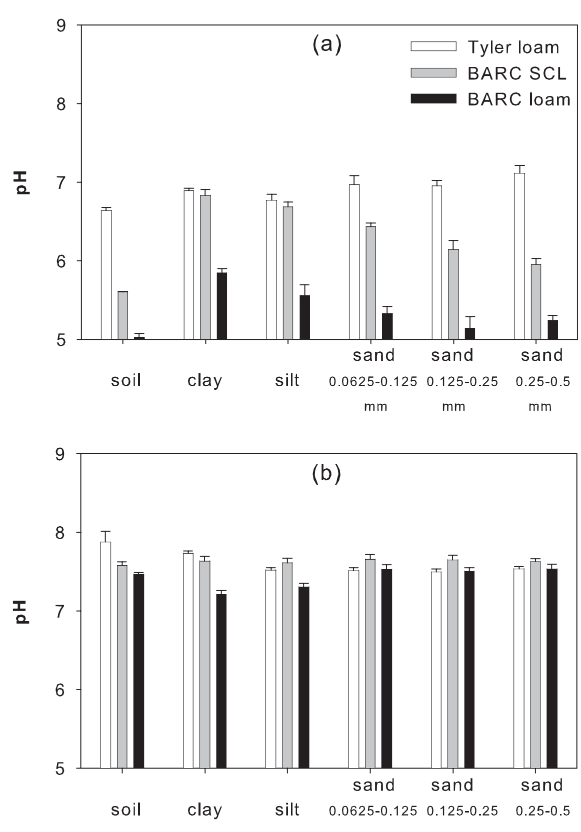

FIG. 2. pH values in soil-water suspensions without (a) and with (b) bovine manure.

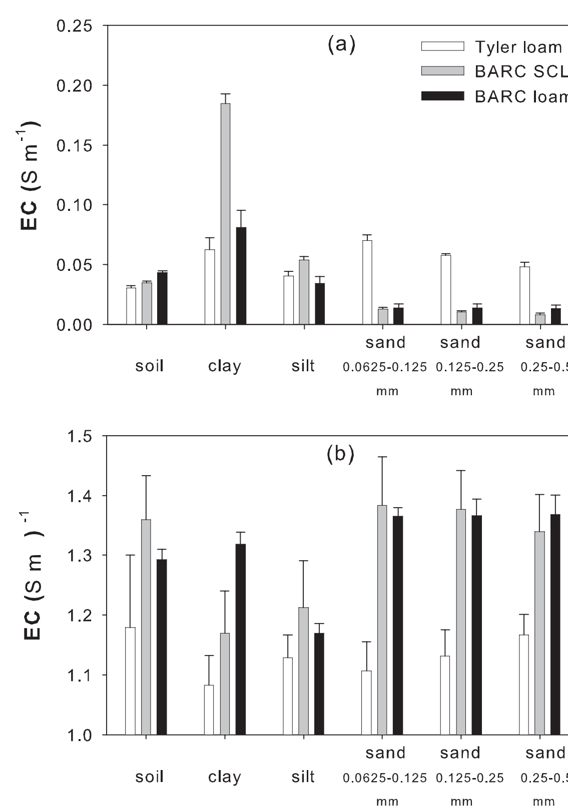

FIG. 3. EC values in soil-water suspensions without (a) and with (b) bovine manure.

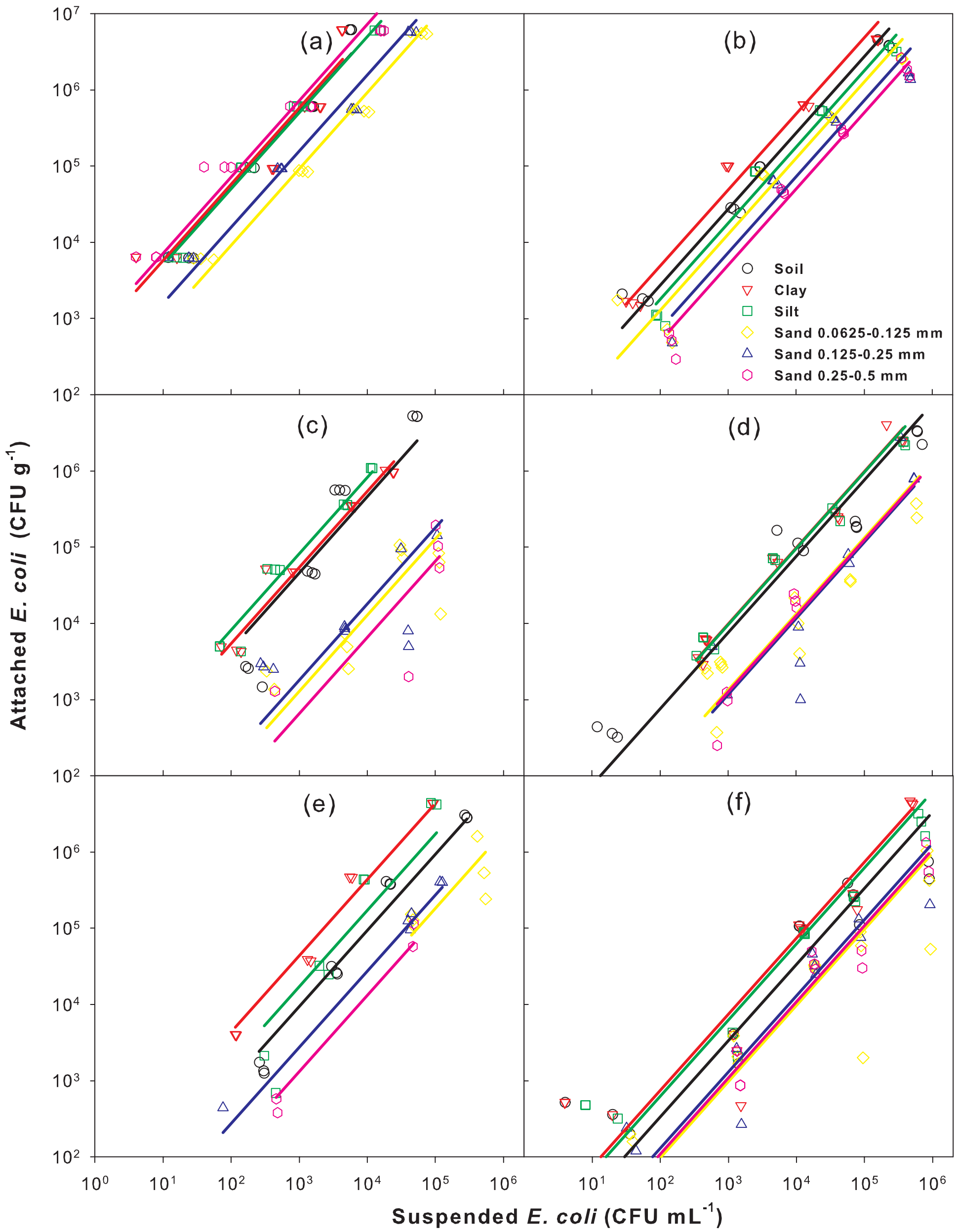

FIG. 4. Relationships between attached amounts of FC and concentrations of FC in suspensions of Tyler loam soil (a, b), BARC SCL (c, d), and BARC loam (e, f); panels a, c, and e show data in the absence of manure, while panels b, d, and f show data in the presence of bovine manure. The lines show the fit with the linear isotherm equation.

The experimental data on FC attachment to soil fractions are shown in Fig. 4. Generally, more FCs were associated with clay and silt fractions than with sand. Relatively more FCs were attached to the Tyler soil fractions than to the BARC soil fractions at the same concentrations in the liquid phase (Fig. 4a, c, and e). This difference was greater in sand fractions than in silt and clay fractions. Manure application considerably decreased the numbers of bacteria attached to soil particles. The most pronounced decrease in numbers of attached FCs occurred in clay and silt fractions (Fig. 4b, d, and f), while a minor decrease was observed in all sand fractions of the BARC SCL and loam soils. In the sand fractions of the Tyler soil, suspended bovine manure decreased the bacterial attachment. However, this attachment was still higher than that for the sand fractions of the BARC soils.

The attachment of bacteria to soils was more similar to the attachment to fine fractions (silt and clay) than to the attachment to sand particles (Fig. 4). The effect of manure on FC attachment to soil was similar to that observed with clay and silt.

The values of K_s obtained with different soil fractions are shown in Table 3. Greater values of K_s were obtained in the soil and clay fractions than in the sand fractions for each soil in both the presence and absence of manure, with the exception

TABLE 3. K_s values for the attachment of FCs to soils and soil fractions in suspensions without and with bovine manure[a]

Soil or soil fraction in suspension:	Tyler loam		BARC SCL		BARC loam	
	K_s (ml g^{-1})	R^2	K_s (ml g^{-1})	R^2	K_s (ml g^{-1})	R^2
Without manure						
Soil	513.2 ± 78.7	0.977	46.3 ± 14.8	0.947	9.40 ± 1.42	0.981
Clay	580.9 ± 145.6	0.894	54.4 ± 6.6	0.961	43.4 ± 6.8	0.980
Silt	501.3 ± 39.8	0.989	81.9 ± 9.1	0.970	17.2 ± 7.7	0.952
Sand, 0.0625–0.125 mm	92.1 ± 11.8	0.981	1.29 ± 0.45	0.692	1.82 ± 0.62	0.582
Sand, 0.125–0.25 mm	156.7 ± 24.2	0.986	1.80 ± 0.84	0.565	2.73 ± 0.46	0.995
Sand, 0.25–0.5 mm	720.5 ± 133.6	0.978	0.65 ± 0.47	0.591	1.31 ± 0.30	0.988
With applied bovine manure						
Soil	27.2 ± 3.4	0.978	7.52 ± 2.10	0.967	3.36 ± 1.70	0.670
Clay	48.6 ± 7.0	0.974	9.82 ± 1.01	0.976	7.44 ± 2.92	0.812
Silt	17.7 ± 2.7	0.972	9.48 ± 1.14	0.983	6.23 ± 1.83	0.886
Sand, 0.0625–0.125 mm	12.8 ± 3.1	0.933	1.33 ± 0.38	0.879	0.96 ± 0.39	0.720
Sand, 0.125–0.25 mm	7.30 ± 1.66	0.882	1.17 ± 0.51	0.777	1.29 ± 0.47	0.836
Sand, 0.25–0.5 mm	5.00 ± 0.65	0.980	1.25 ± 0.36	0.953	1.09 ± 0.22	0.952

[a] K_s values are averages ± standard deviations estimated from triplicate experiments. R^2, coefficient of determination for the linear isotherm equation.

of the sand fraction of 0.2- to 0.5-mm particle size of the Tyler soil (Table 3). The average ratios of $K_{s(\mathrm{clay})}/K_{s(\mathrm{sand})}$ were 22 and 44 and the average ratios of $K_{s(\mathrm{silt})}/K_{s(\mathrm{sand})}$ were 9 and 66 for BARC loam and BARC SCL, respectively; these results were comparable to Karpinskaya's results (quoted in reference 27). However, such differences were not so pronounced for the Tyler loam soil, where the ratios of $K_{s(\mathrm{clay})}/K_{s(\mathrm{sand})}$ and $K_{s(\mathrm{silt})}/K_{s(\mathrm{sand})}$ were 1.8 and 1.6, respectively. For the BARC soils and soil fractions with and without manure, the differences between the K_s values for the clay and silt fractions were less than the differences between the K_s values of the sand fractions and fine (clay and silt) fractions. For the Tyler soil, the differences between the K_s values of different fractions were much less than for the BARC soils. The highest values of K_s in soil fractions without manure were obtained for the silt and clay fractions of the Tyler soil; the lowest values were obtained for the sand fractions of the BARC SCL soil (Table 3).

The differences in K_s between the BARC and Tyler soils correlated with the differences in the OC contents. The OC content in the sand fractions was from 18 to 124 times less than in the silt and the clay fractions of the BARC loam and from 131 to 388 times less than in the same fractions of the BARC SCL soil, whereas the OC contents in the sand fractions of the Tyler soil were 1.7 and 1.4 times higher than those in the silt and clay fractions (Table 2). Organic matter in sandy soils was found to be important for microbial attachment by Gray et al. (18), who reported that about 60% of *Phytophthora* spp. in a sand dune soil were located on organic particles, which represented only 15% of the available (colonizable) soil surface. They calculated that only 0.02% of the available surface area of the sand grains was colonized by bacteria. The much-stronger attachment of FCs to the sand fraction of the Tyler soil as compared with that for other sand fractions seems to be related to the surface coating containing large amounts of organic matter.

The introduction of manure in the bacterial suspensions caused a considerable decrease in the values of K_s in the clay and silt fractions (Table 3). For example, K_s in the clay and silt fractions of the BARC SCL soil decreased from 46 to 82 ml g^{-1} to 8 to 10 ml g^{-1}. The decrease in the K_s values was much less or not noticeable at all in the sand fractions of the BARC soils. For example, in the sand fractions, the values of K_s were in the ranges of 0.7 to 1.8 ml g^{-1} and 1.2 to 1.3 ml g^{-1} for the BARC SLC without and with manure treatment, respectively, and in the ranges of 1.3 to 2.7 ml g^{-1} and 1.0 to 1.3 for the BARC loam without and with manure treatment, respectively. The effect of manure on FC attachment was substantial for the sand fractions of the Tyler soil, where the values of K_s were from 7 to 144 times less in the soil-manure suspension than in the soil-water suspension, and the difference in K_s increased with the increase in the sand fraction particle size.

The bovine manure used in our study was a complex matrix consisting of microbial biomass, dietary fiber, bedding materials, urine, and fecal mucus. Because of this complexity, known factors of bacterial attachment to mineral surfaces might work in the same way or in a completely different way in comparison to attachment in suspensions without manure. An increase of the electrolyte concentration is known to increase bacterial attachment to solid particles (16, 28, 29, 38, 39). EC increased with manure addition in our experiments, and yet bacterial attachment markedly decreased. The solution pH has also been reported to affect bacterial attachment to mineral surfaces, with the strongest adsorption of bacterial cells occurring at pH values in the range from 3 to 6 (10, 37). Reddy et al. (35) and Scholl and Harvey (38) showed that the fraction of bacteria retained by the soil decreased markedly as the pH was increased above 6. The manure suspensions had pH values higher than those of the bacterium-water suspensions in our study (Fig. 2), and the FC attachment to soil and fine particles in response to the increase in pH was similar to that reported in the studies carried out by other authors in the absence of manure in the suspensions. The dissolved organic matter was shown to modify the surfaces of bacteria and mineral particles and thus decrease the attachment (9). Dissolved and suspended colloidal organic matter also decreased the bacterial attachment by competing with bacteria for the same attachment sites (38, 45). Finally, as bacterial attachment to both soil and manure particles may occur (30, 31), the number of bacteria attached to the soil surface can also be reduced due to cell competition for attachment sites on soil and on manure par-

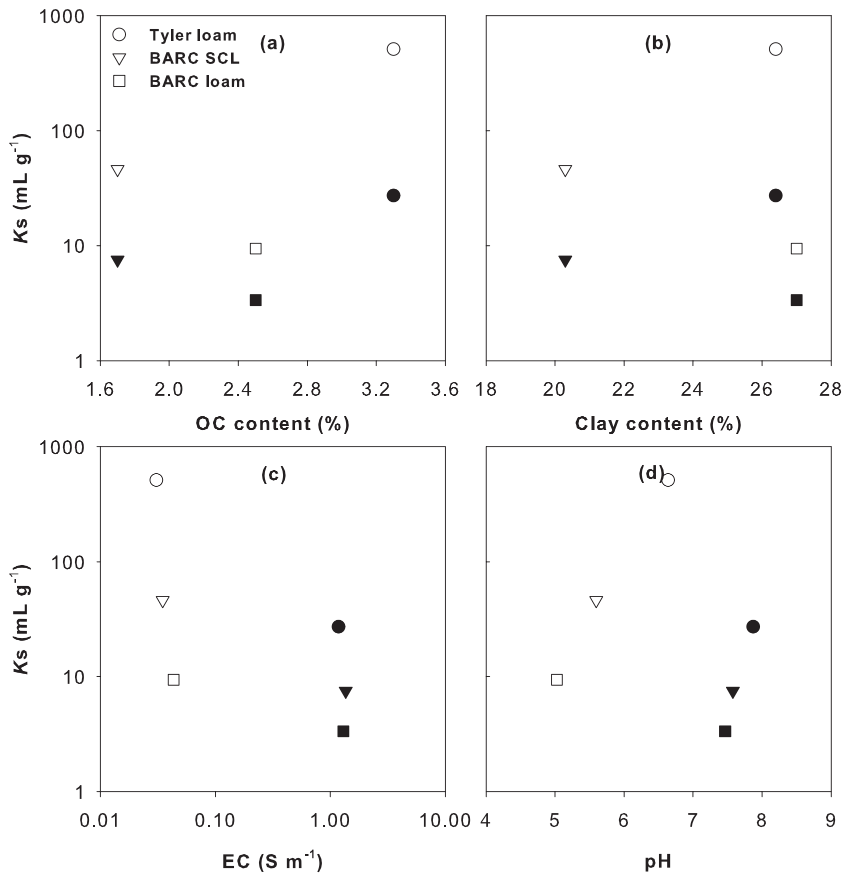

FIG. 5. Relationships between K_s values, soil OC contents, soil clay contents, and EC and pH values in suspensions. The hollow and filled symbols show data from suspensions without and with manure, respectively.

ticulates. In our work, the effect on bacterial attachment of manure in the suspensions was similar to the effect on bacterial attachment of organic materials in suspension reported in the works of other authors. Due to the many interacting factors affecting bacteria and solid surface properties in the bacterium-manure solution, we cannot conclusively identify the dominant mechanism by which added manure decreased FC attachment to soil particles.

The linear attachment isotherm equation described the attachment reasonably well, with R^2 (coefficient of determination) in the range from 0.80 to 0.98 for two of three soils, all clay fractions, and all silt fractions in both the absence and presence of manure (Table 3). The goodness of fit of the equation was worse for sand fractions where the attachment was low and the nonlinearity of the attachment isotherm was pronounced (Fig. 4).

The values of K_s for soil samples without separation into fractions are plotted against the pH, EC, OC, and clay content values in Fig. 5. We did not observe the increase in adherence of bacteria to soil with the increase of clay content which was documented by other authors (19, 26, 46). Gromyko et al. (19), for example, reported an increase in K_s from 73.2 to 5,546 ml g^{-1} as the clay content increased from 26 to 66% for Serozem soils. Ling et al. (26) derived the regression equation ln (K_s) = 3.9 ln (clay%) − 11.3 for clay contents in the range of 10 to 54%. A possible reason for the absence of the dependence of K_s on clay content in our work is the narrow range of clay contents in the three studied soils (Fig. 5). K_s was distinctly less in the manure suspension for all soils regardless of soil OC and clay contents (Fig. 5a and b). The values of K_s for soils decreased about 19-, 6-, and 3-fold after manure was added to the suspensions of Tyler loam, BARC SCL, and BARC loam soil, respectively. Greater values of K_s were obtained at smaller values of pH and EC in the suspension for each soil sample (Fig. 5c and d). The increase in K_s was correlated more to increasing soil pH, in the ranking order BARC loam < BARC SCL < Tyler loam soil, than to the other factors.

Although a large number of bacterial adsorption studies have been published in the last decade, quantitative information on the values of K_s for manure-borne bacteria in different soils is still limited. Therefore, for purposes of comparison, we derived K_s values from the results of previously published experiments. The calculated values of K_s varied greatly in different soils, i.e., they ranged from 33 to 9,900 ml g^{-1} for Serozem soils of central Asia (19) and were 0.7, 16.7, 88.2, and 9,790 ml g^{-1} in Arenosa loamy sand, San Angelo SCL, Houston Black clay, and Beaumont clay soils, respectively (46). Ling et al. (26) obtained K_s values of 0.33 and 127 ml g^{-1} in Tangi silt loam and Commerce clay loam, respectively. In our study, the values of K_s were 9.4, 46.3, and 513.2 ml g^{-1} for the BARC loam, BARC SCL, and Tyler loam soil, respectively. The range of K_s

values in this study had an overlap with the ranges found by other authors.

The values of K_s obtained in this study indicate that substantial amounts of bacteria can attach to suspended sediment, provided that the batch values of K_s are applicable in the dynamic conditions of nonstationary overland flow. Therefore, the best management practices that decrease the amount of sediment reaching waterways and water bodies should also be efficient in the retention of the manure-borne bacteria. The importance of bacterial attachment to suspended solids depends on the amount of sediment generated during the runoff event. Roodsari et al. (36) reported the results of rainfall simulations with manure applied on the top of either bare or vegetated 20% slopes. About 20% of manure-borne bacteria released on bare slopes were found in suspended solids in runoff at the bare plots, whereas no appreciable amounts of suspended solids were found in the runoff from the vegetated plots (36).

Recent reviews (13, 44) have highlighted a lack of quantitative understanding of the status of suspended bacteria in overland flow, i.e., whether they are attached to soil or fecal particles or move as unattached cells or bacterial aggregates. Although more experimental information is needed, the results of our work suggest that one approach to mitigating the transport of manure-borne bacteria to surface waters is to control the amount of sediment reaching the water.

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